Project 1025341

Microbial pathways for the mobilization of mercury as Hg(O) in anoxic subsurface environments

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RESULTS TO DATE: The goal of our project which was initiated in June 2005 is focused on the presence of merA in microbial communities of anoxic environments and the effect of anaerobic respiratory pathways on MR expression and activities. The following progress has been made to date:

PCR primers were designed to span the known phylogenetic range of merA genes of Gram-negative bacteria. In control experiments, these primers successfully amplified a 288 bp region at the 3? end of previously characterized merA genes from Shewanella putrefaciens pMERPH, Acidithiobacillus ferrooxidans, Pseudomonas stutzeri pPB, Tn5041, Pseudomonas sp. K-62, and Serratia marcescens pDU1358.

The abundance and diversity of merA were assessed in anaerobic enrichments from Berry?s Creek, a highly industrially contaminated site in the Meadowlands, NJ, by sequencing a merA clone library obtained by PCR from sediment DNA extracts. Anaerobic sediment slurries were supplemented with two additions of 10 microgram Hg(II)/g and incubated for 3 weeks at which time the DNA was isolated and PCR amplified. The amplicons were TOPO cloned into pCR2.1 (Invitrogen) and sequenced. A total of 79 clones were sequenced of which 69 represented unique amplicons. The sequences were aligned and a phylogenetic tree was built. While many sequences aligned with previously described merA genes from both Gram positive and Gram negative isolates, five novel lineages (I through V), were identified. These results reveal a previously unrecognized diversity of merA and suggest that bacterial activities may play a role in mercury reduction in anaerobic environments.

Anaerobic enrichments of Meadowlands sediments were set up for the purpose of isolating pure cultures carrying novel merA genes. Five oligoprobes specific for each of the novel merA clusters were designed, tested, and hybridized with genomic DNA of Hg(II) resistant isolates from the enrichments. Three strains, a Bacillus sp. and a Streptomyces sp. with merA of cluster V, and a Pseudomonas sp. with merA of cluster II, were isolated from the fermentative enrichment. One denitrifying isolate, a Paenibacillus sp. carried a cluster III merA. The entire mercury resistance systems in these strains are currently being examined genetically and biochemically to fully characterize the mechanisms by which anaerobic bacteria interact with mercury.

During the coming year we plan to expand these studies by setting up enrichments of subsurface sediments from mercury contaminated and control aquifers. The enrichments will be carried out under various terminal oxidation conditions and the rates of mercury reduction will be determined. Pure culture isolation and metagenomic studies will be carried out on these enrichments to identify the organisms responsible for the observed transformations.

DELIVERABLES: Ni Chadain, S., J.K. Schaefer, S. Crane, G.J. Zylstra, and T. Barkay. Comparative analysis of mercuric reductase (merA) gene diversity in mercury-contaminated water and sediment. Env. Microbiol. Submitted